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A Study on Coronary Hemodynamics During Acetylcholine-Induced Coronary Spasm in Patients With Variant Angina: Endothelium-Dependent Dilation in the Resistance Vessels

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The epicardial coronary artery of patients with variant angina is hyperreactive to the constrictive effect of acetylcholine, but it is not known whether the coronary microvasculature also constricts in response to acetylcholine. Incremental doses of acetylcholine were injected into the left coronary artery of 57 patients with variant angina and with spasm in this artery. By measuring coronary sinus blood flow, coronary hemodynamic status just before angiographic documentation of spasm was examined.

Acetylcholine induced spasm in the left coronary artery in all patients. It also decreased the diameter of the nonspasm artery by 36 ± 19% from baseline. For all patients, coronary sinus blood flow was 89 ± 38 ml/min at baseline and increased to 104 ± 61 ml/min during an acetylcholine-induced anginal attack ($p < 0.01$). In 10 patients with spasm in both the left anterior descending and left circumflex arteries (that is, multivessel spasm), coronary sinus blood flow decreased from 84 ± 21 to 52 ± 26 ml/min ($p < 0.01$). In the other 47 patients with spasm in only one of these two arteries (that is, single-vessel spasm), coronary sinus blood flow increased from 90 ± 41 to 115 ± 61 ml/min ($p < 0.01$) without change in the rate-pressure product.

It is concluded that in patients with variant angina, acetylcholine induces spasm and constriction in the epicardial coronary artery, whereas it dilates the resistance vessels presumably through the release of the endothelium-dependent relaxing factor.

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Variant angina is caused by an absolute reduction in regional myocardial blood flow due to spasm of an epicardial coronary artery (1,2). Coronary blood flow has been demonstrated to decrease before a spontaneous ischemic attack in patients with variant angina (3,4). Administration of ergonovine maleate, which is frequently used as a provocative test for coronary spasm in studies of the pathogenesis of angina pectoris (5–7), has been shown to cause a reduction in both total and regional coronary blood flow during the induced attack in patients with variant angina (8,9).

We have shown (10,11) that acetylcholine, an endothelium-dependent vasodilator, induces spasm of an epicardial coronary artery in >90% of patients with variant angina but not in patients without angina at rest and without significant coronary artery disease. In patients with variant angina, therefore, the epicardial coronary vascular endothelium seems to be impaired or the coronary vascular smooth muscle is hyperreactive to a vasoconstricting effect of acetylcholine, or both. However, we have also shown (12) that acetylcholine causes a significant increase in the coronary blood flow in humans with normal or nearly normal coronary arteriograms, and this finding suggests that acetylcholine induces vasodilation in the coronary resistance vessels, presumably by releasing the endothelium-derived relaxing factor. Other investigators (13–15) have subsequently reported a similar finding. It is not known, however, whether the coronary resistance vessels of patients with variant angina dilate in response to acetylcholine. In the present study, the effects of acetylcholine on coronary hemodynamics were examined by measuring coronary sinus blood flow in patients with variant angina and spasm in the left coronary artery.

Methods

Study patients. The study group comprised 57 patients with variant angina in whom spasm of the left coronary artery (that is, total or subtotal occlusion or severe constriction associated with ischemic electrocardiographic [ECG] changes or chest pain, or both) was induced with intracoronary injection of acetylcholine. There were 52 men and 5 women with a mean age of 56 years (range 38 to 77). All patients showed transient ST segment elevation on the ECG

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Table 1. Clinical and Angiographic Characteristics of 10 Patients in the Multivessel Spasm Group

Pt No.	Age (yr)/ Gender	ACh Dose (μg)	Site and Degree of Coronary Spasm	% Stenosis in LCA After Nitroglycerin
1	46/M	50	LAD (6), total occlusion LCx(11), total occlusion	Normal
2	69/M	20	LAD (6), total occlusion LCx(11), total occlusion	S6 25%
3	50/M	20	LAD (6), total occlusion LCx(11), total occlusion	Normal
4	57/M	20	LAD (6), subtotal occlusion LCx(13), total occlusion	Normal
5	65/M	50	LAD (7), total occlusion LCx(13), subtotal occlusion	S7 90%, S11 50%
6	55/M	20	LAD (6), total occlusion LCx(12), total occlusion	S6 25%, S13 25%
7	67/M	100	LAD (7), total occlusion LCx(12), subtotal occlusion	S7 25%, S12 25%
8	52/F	100	LAD (7), subtotal occlusion LCx(12), subtotal occlusion	S6 50%, S7 50%
9	48/M	50	LAD (9), total occlusion LCx(11), total occlusion	Normal
10	50/M	50	LAD (9), total occlusion LCx(13), total occlusion	S6 25%

Sites of coronary spasm (numbers in parentheses) and fixed stenosis in the left coronary artery (LCA) after nitroglycerin: S6 to S13 refer to the segments of the coronary arteries as defined by the American Heart Association (AHA) committee report. A reporting system on patients evaluated for coronary artery disease. Circulation 1975;51:5–40. Ach = acetylcholine; F = female; LAD = left anterior descending coronary artery; LCx = left circumflex coronary artery; M = male; Pt = patient.

during a spontaneous episode of angina or during an anginal attack induced by hyperventilation or treadmill exercise testing, or both. They had a mean serum cholesterol level of 191 ± 36 mg/dl (range 115 to 280); only two patients had a level >250 mg/dl. The coronary arteriographic findings after administration of nitroglycerin (0.3 mg) are shown in Tables 1 and 2.

In another group of 10 control patients without angina pectoris and with normal coronary arteriograms or minimal coronary artery disease (≤25% stenosis of lumen diameter), the time course of the effect of intracoronary injection of acetylcholine on coronary sinus blood flow was studied. This control group comprised nine men and one woman with a mean age of 53 years. These patients underwent diagnostic cardiac catheterization for the evaluation of atypical chest pain (nine patients) or abnormal, inverted T waves on the ECG (one patient).

All antianginal drugs were withdrawn at least 3 days before the study except sublingual nitroglycerin, which was also withdrawn 6 h before the study. No patient had congestive heart failure, allergy, active peptic ulcer, chronic obstructive lung disease or any other serious disease. Written informed consent was obtained from all patients before the study. The study was in agreement with the guidelines approved by the ethics committee at our institution.

Table 2. Clinical and Angiographic Characteristics of 47 Patients in the Single-Vessel Spasm Group

Pt No.	Age (yr)/ Gender	ACh Dose (μg)	Site and Degree of Coronary Spasm	% Stenosis in LCA After Nitroglycerin
11	38/M	100	LAD (6), total occlusion	Normal
12	56/M	50	LAD (6), total occlusion	Normal
13	51/M	50	LAD (6), total occlusion	S6 50%
14	72/M	50	LAD (6), total occlusion	S6 50%, S11 25%
15	41/M	20	LAD (7), total occlusion	S7 25%
16	49/F	100	LAD (7), subtotal occlusion	Normal
17	59/M	50	LAD (6), subtotal occlusion	S13 25%
18	59/M	50	LAD (8), total occlusion	S7 75%
19	54/M	50	LAD (6), subtotal occlusion	S6 75%, S13 50%
20	58/M	100	LAD (7), total occlusion	S7 50%
21	45/M	50	LAD (6), subtotal occlusion	S6 25%, S11 25%
22	59/F	100	LAD (7), total occlusion	S7 90%
23	49/M	100	LAD (7), total occlusion	S7 90%
24	48/M	100	LAD (7), total occlusion	S7 25%
25	52/M	100	LAD (6), total occlusion	S6 90%
26	46/M	100	LAD (7), subtotal occlusion	S7 90%
27	45/M	100	LAD (6), subtotal occlusion	Normal
28	46/M	100	LAD (7), subtotal occlusion	Normal
29	55/M	20	LAD (7), total occlusion	S7 25%
30	63/F	100	LAD (6), total occlusion	S6 90%
31	63/M	50	LAD (7), total occlusion	S7 75%
32	43/M	20	LAD (6), total occlusion	S7 25%
33	55/M	20	LAD (7), total occlusion	S7 25%, S11 25%
34	65/M	50	LAD (7), subtotal occlusion	S7 50%
35	61/M	20	LAD (7), total occlusion	S7 90%
36	54/M	50	LAD (7), total occlusion	S7 75%
37	61/M	50	LAD, diffuse constriction	Normal
38	68/M	50	LAD, diffuse constriction	Normal
39	57/M	100	LAD, diffuse constriction	Normal
40	58/M	100	LAD, diffuse constriction	Normal
41	64/F	100	LAD, diffuse constriction	Normal
42	61/M	100	LAD, diffuse constriction	S7 50%
43	62/M	100	LAD, diffuse constriction	S6 25%
44	57/M	50	LAD, diffuse constriction	Normal
45	66/M	100	LAD, diffuse constriction	S7 50%
46	42/M	50	LAD (9), total occlusion	Normal
47	50/M	50	LAD (9), total occlusion	Normal
48	56/M	20	LAD (9), subtotal occlusion	Normal
49	64/M	100	LAD (9), subtotal occlusion	S9 75%
50	49/M	50	LAD (9), subtotal occlusion	Normal
51	56/M	100	LCx(11), subtotal occlusion	Normal
52	70/M	50	LCx(13), total occlusion	S8 50%, S11 25%
53	77/M	100	LCx(11), subtotal occlusion	S7 50%, S13 75%
54	50/M	100	LCx(11), total occlusion	S7 25%, S11 50%
55	62/M	100	LCx(11), total occlusion	S13 25%
56	62/M	50	LCx(13), total occlusion	S13 50%
57	60/M	50	LCx(13), total occlusion	S7 25%

Footnote and abbreviations as in Table 1.

Cardiac catheterization. The study was performed in the morning while the patients were in the fasting state. A 7F thermodilution coronary blood flow catheter (CCS-7U-90B, Webster) was positioned in the coronary sinus by way of the right antecubital vein. The catheter position was determined by injection of a small volume of contrast medium (Hexabrix

320), and stable catheter position was confirmed by fluoroscopy during the procedure. A 6F Goodale-Lubin catheter (USCI) for blood sampling was also inserted from the right antecubital or right subclavian vein and positioned in the coronary sinus in the same way. Coronary arteriography was performed with the Sones technique, and control coronary arteriograms of the left coronary artery in the right anterior oblique projection and of the right coronary artery in the left anterior oblique projection were taken. Relations between the focal spot, the patient and the height of image tube were kept constant. A tripolar electrode catheter (USCI) was inserted into the right ventricular apex by way of the femoral vein and connected to a temporary pacemaker set at a rate of 40 to 50 beats/min. Three ECG leads (I, aVF and V_3 or V_4) and arterial blood pressure were continuously monitored on an oscilloscope during the study. Six ECG leads (I, II, aVF, V_1, V_3 and V_5) were continuously recorded, and 12 ECG leads were recorded at appropriate intervals.

Coronary sinus blood flow was determined by the injection of nonheparinized normal saline solution through the thermodilution catheter with a constant infusion pump at a rate of 36 ml/min and calculated with use of a Thermo Flow RF (Good Man) (16). The flow curve was recorded on oscillographic paper, along with three ECG leads and arterial pressure.

Blood samples for plasma lactate concentration were collected from the aortic root and coronary sinus, placed in chilled glass tubes containing ethylenediamine tetraacetic acid (EDTA)-2Na solution and centrifuged at 4°C; the plasma was then separated. The plasma lactate concentration was determined by an enzymatic method using Determiner LA (Kyowa Medex).

Study Protocol

Control patients. After measurement of baseline aortic pressure, heart rate and coronary sinus blood flow, 100 μg of acetylcholine was injected into the left coronary artery for 20 s, and the same measurement was repeated every minute for 5 min. The same dose of acetylcholine was again injected into the left coronary artery, and the left coronary arteriogram was taken 1 min after acetylcholine injection.

Patients with variant angina group. After measurement of baseline aortic pressure, heart rate and coronary sinus blood flow, incremental doses of acetylcholine (20, 50 and 100 μg) were injected into the left coronary artery until coronary spasm was induced or the maximal dose (100 μg) was reached (10,11). The injection of each dose of acetylcholine was performed for 20 s, and the time interval between each injection was 5 min. Sixty to 70 s after initiation of the injection of each dose of acetylcholine (that is, 40 to 50 s after completion of the injection), aortic pressure, heart rate and coronary sinus blood flow were measured, and then the left coronary arteriogram was taken. The timing of the measurement of coronary sinus blood flow after acetylcholine injection was determined on the basis of our previous observation (10,11) that coronary spasm was induced 1 min after completion of acetylcholine injection in most patients with variant angina and on the present observation in control patients that the maximal increase in coronary sinus blood flow after acetylcholine injection occurred 1 min after initiation of the injection (Fig. 1).

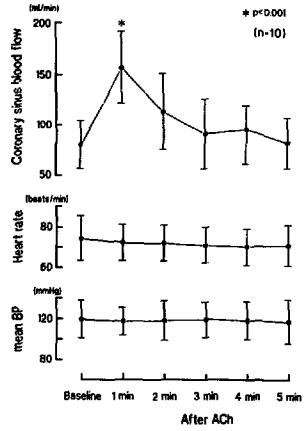

Figure 1. Time course of the changes in coronary sinus blood flow, heart rate and mean blood pressure (BP) after injection of 100 μg of acetylcholine (ACh) in 10 control subjects. Coronary sinus blood flow increased after acetylcholine injection and reached its peak 1 min after the injection; neither heart rate nor mean blood pressure changed after the injection.

When spasm of the left coronary artery was confirmed by arteriography, blood samples were collected from the aortic root and coronary sinus for measurement of myocardial lactate extraction ratio. When spasm induced in the left coronary artery resolved spontaneously without use of nitroglycerin, incremental doses of acetylcholine (20 and 50 μg) were then injected into the right coronary artery in the same way. When induced coronary spasm did not resolve spontaneously within 5 min or anginal chest pain persisted for ≥2 min or hemodynamic instability due to ischemia developed, 100 to 200 μg of nitroglycerin was injected into the coronary artery involved. In all patients, the arteriograms of the left and right coronary arteries were taken from multiple projections after administration of nitroglycerin.

Quantitative coronary arteriography. To assess the change in coronary artery lumen diameter after injection of acetylcholine, the lumen diameter was measured before and after acetylcholine with the use of a computer-assisted

coronary angiography analysis system. End-diastolic cinefilms were videodigitized and stored in the cardiac image analysis system (Cardio 500, Kontron Instruments). Automated contour detection was performed by a geometric edge differentiation technique similar to that described by Reiber et al. (17). Details of the method of quantitative coronary angiography were described in our previous study (18), and the technique was validated. The overall accuracy and precision of this method were 2.2 ± 3.3% and 4.6 ± 2.5%, respectively.

Measurements were performed by two investigators. Whenever they considered a segment of the automatically detected contour to be inaccurate, they discussed the proper positions and corrected them accordingly by interacting with the cursor. Analysis of intra- and interobserver variability for the measurement of lumen diameter showed high reproducibility ($r = 0.99$, SEE = 0.05 mm, $p < 0.001$ and $r = 0.99$, SEE = 0.04 mm, $p < 0.001$, respectively). Diameter was measured at the midsegments of the left anterior descending and left circumflex arteries in the control subjects and at the site of greatest change in diameter after acetylcholine injection in patients with variant angina.

Calculation and data analysis. Coronary vascular resistance (mm Hg · min per liter) was calculated as Mean aortic blood pressure × 1,000/Coronary sinus blood flow. Rate-pressure product (mm Hg · beats/min) was calculated as Heart rate × Systolic blood pressure. Myocardial lactate extraction ratio (%) was calculated as 100 × (Arterial lactate concentration − Coronary sinus lactate concentration)/Arterial lactate concentration.

All data are shown as mean values ± 1 SD. The time course of the changes in aortic pressure, heart rate and coronary sinus blood flow after acetylcholine injection in the control patients was statistically analyzed with an analysis of variance with a subsequent analysis using Bonferroni *t* test. The hemodynamic variables and coronary sinus blood flow before and after acetylcholine injection in patients with variant angina were compared with a paired *t* test. The patients with variant angina were classified into those with multivessel coronary spasm and those with single-vessel spasm. The changes in the coronary and systemic hemodynamic variables after acetylcholine injection were compared among these two groups and control patients with an analysis of variance. A p value < 0.05 was considered significant.

Results

Time course of the effect of acetylcholine in control subjects. Figure 1 shows the time course of the changes in coronary sinus blood flow, heart rate and mean arterial pressure after acetylcholine injection (100 μg) into the left coronary artery in 10 control subjects. Coronary sinus blood flow, which was 80 ± 24 ml/min at baseline, increased and reached a peak (156 ± 35 ml/min, $p < 0.001$ vs. baseline) 1 min after the injection and then returned to the baseline. Heart rate transiently decreased during acetylcholine injection in some patients but returned to the baseline value immediately after the injection. Both heart rate and mean arterial pressure measured at 1-min intervals after acetylcholine injection remained unchanged. The lumen diameter of the left anterior descending and left circumflex arteries decreased after acetylcholine injection by 11 ± 6% ($p < 0.01$) and 10 ± 13% ($p < 0.05$), respectively.

Coronary Spasm Induced by Acetylcholine in Patients With Variant Angina

Of the 57 patients with variant angina, acetylcholine induced coronary spasm in the left anterior descending artery or in its branch in 40, in the left circumflex artery in 7 and in both of these arteries simultaneously in the remaining 10. The patients were classified into two groups: 10 patients with multivessel coronary spasm and 47 patients with single-vessel spasm.

Multivessel spasm group (Table 1). Total occlusion was simultaneously induced at the origin of the left anterior descending and left circumflex arteries in three patients. In the remaining seven patients, total occlusion or subtotal occlusion (defined as focal occlusion with a filling delay of the contrast medium) of the left anterior descending artery (five patients) or severe constriction of the left anterior descending artery with concomitant total occlusion of the diagonal branch (two patients) was induced with concomitant total or subtotal occlusion of the circumflex artery or the obtuse marginal artery.

Single-vessel spasm group (Table 2). Total or subtotal occlusion was induced in the left anterior descending artery in 26 patients, in the diagonal branch in 5 and in the circumflex artery in 7. Severe, diffuse constriction of the left anterior descending artery with a diameter reduction of 80 ± 7% from baseline was induced in the remaining nine patients. In the 40 patients with spasm in the left anterior descending artery or in its branch, a moderate degree of vasoconstriction with a diameter reduction of 36 ± 19% from baseline was induced in the left circumflex artery (nonspasm artery) after acetylcholine injection. In the seven patients with spasm in the left circumflex artery, vasoconstriction with a diameter reduction of 41 ± 19% from baseline was induced in the left anterior descending artery (nonspasm artery) after acetylcholine injection. The diameter changes of these nonspasm arteries after acetylcholine injection were both significantly greater than those seen in control subjects ($p < 0.001$ for the left circumflex artery and $p < 0.005$ for the left anterior descending artery).

One or more significant stenotic lesions were present in the left coronary artery in 1 patient with multivessel spasm and in 12 with single-vessel spasm. The dose of acetylcholine used to induce coronary spasm is shown in Tables 1 and 2 for each patient.

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Table 3. Changes in Coronary and Systemic Hemodynamics After Acetylcholine Administration in 57 Patients With Variant Angina

	CSBF (ml/min)		CVR (mm Hg·min per liter)		Heart Rate (beats/min)		Systolic BP (mm Hg)		Rate-Pressure Product (mm Hg·beats per min)		LER (%)	
	Baseline	ACh	Baseline	ACh	Baseline	ACh	Baseline	ACh	Baseline	ACh	Baseline	ACh
Multivessel spasm (n = 10)	84 ± 21	52 ± 26*	1,332 ± 329	2,421 ± 1,734*	73 ± 14	73 ± 10	155 ± 27	129 ± 24*	11,356 ± 3,562	9,295 ± 1,793	37 ± 16	−47 ± 102†
Single-vessel spasm (n = 47)	90 ± 41	115 ± 61*	1,352 ± 501	1,120 ± 522*	72 ± 13	72 ± 13	144 ± 23	140 ± 25	10,378 ± 2,768	10,150 ± 2,553	39 ± 17	−20 ± 55*
Total (n = 57)	89 ± 38	104 ± 61*	1,357 ± 467	1,357 ± 984	72 ± 13	72 ± 13	145 ± 24	138 ± 25*	10,549 ± 2,912	9,997 ± 2,443†	39 ± 17	−23 ± 63*

*p < 0.01 versus baseline; †p < 0.05. ACh = acetylcholine; BP = blood pressure; CSBF = coronary sinus blood flow; CVR = coronary vascular resistance; LER = lactate extraction ratio.

Figure 2. Changes in coronary sinus blood flow after injection of acetylcholine (ACh) in 57 patients with variant angina. Acetylcholine injection resulted in coronary spasm in the left coronary artery.

Coronary and Systemic Hemodynamics During Induced Spasm (Table 3)

For all 57 patients with variant angina, mean coronary sinus blood flow, which was 89 ± 38 ml/min at baseline, significantly increased to 104 ± 61 ml/min ($p < 0.01$) 1 min after acetylcholine injection, which resulted in the induction of spasm (Fig. 2). Coronary vascular resistance and heart rate remained unchanged. Systolic arterial pressure and rate-pressure product decreased significantly after acetylcholine ($p < 0.01$ and $p < 0.05$, respectively). Myocardial lactate extraction ratio decreased significantly after acetylcholine ($p < 0.01$).

Single-vessel versus multivessel spasm. The changes in coronary and systemic hemodynamics were analyzed for each group (Table 3). In the multivessel spasm group, coronary sinus blood flow decreased in all patients. Mean coronary sinus blood flow, which was 84 ± 21 ml/min at baseline, decreased significantly to 52 ± 26 ml/min after acetylcholine injection ($p < 0.01$) (Fig. 3). In the single-vessel spasm group, mean coronary sinus blood flow, which was 90 ± 41 ml/min at baseline, increased significantly to 115 ± 61 ml/min after acetylcholine ($p < 0.01$). The patients in this group were classified according to the spasm site: in the 40 patients with spasm in the left anterior descending artery or in its branch, coronary sinus blood flow increased from 94 ± 43 to 117 ± 66 ml/min ($p < 0.01$) after acetylcholine; in the 7 patients with spasm in the circumflex artery, it increased from 70 ± 6 to 108 ± 22 ml/min ($p < 0.01$) after acetylcholine (Fig. 4). Percent changes in coronary sinus blood flow after acetylcholine were compared among control patients and patients in the multivessel and single-vessel spasm groups (Fig. 5). There were significant differences among the changes in all three groups (all $p < 0.001$).

Coronary vascular resistance. This variable increased in all but one patient in the multivessel spasm group ($p < 0.01$). In contrast, it decreased significantly in the single-vessel spasm group ($p < 0.01$): in the 40 patients with spasm in the

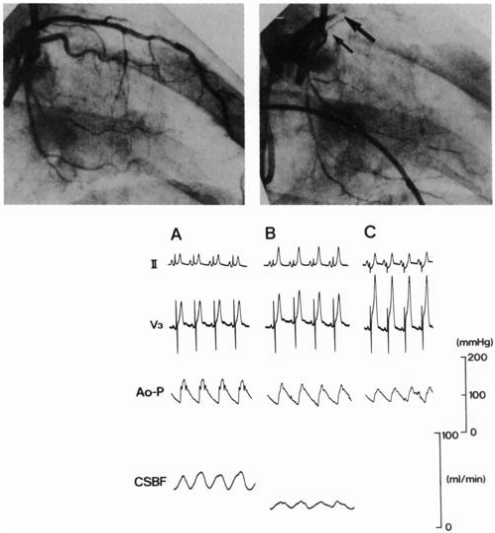

Figure 3. Top, Left coronary arteriograms taken before (**left**) and after (**right**) injection of 20 μg of acetylcholine in Patient 6 with multivessel coronary spasm. Total occlusion was induced in the left anterior descending artery (**large arrow**) and obtuse marginal artery (**small arrow**). **Bottom,** Electrocardiographic leads II and V_5, aortic pressure (Ao-P) and coronary sinus blood flow (CSBF) recorded at baseline (**A**) and 1 min after injection of acetylcholine (**B**) in the same patient. After injection of acetylcholine, mean coronary sinus blood flow decreased from 58 to 24 ml/min. Marked ST segment elevation in lead V_5 was noted just before arteriography (**C**).

left anterior descending artery or its branch, coronary vascular resistance decreased from 1,344 ± 542 to 1,162 ± 555 mm Hg · min per liter ($p < 0.01$) after acetylcholine injection; in the other 7 patients with spasm in the circumflex artery, it decreased from 1,396 ± 133 to 882 ± 148 mm Hg · min per liter ($p < 0.01$) after acetylcholine. Percent changes in coronary vascular resistance after acetylcholine in control subjects and in the two patient groups with variant angina were compared (Fig. 5). The change in the multivessel spasm group was significantly different from that in the single-vessel spasm group and in control patients (both $p < 0.001$). There was no statistical difference between the change in the single-vessel spasm group and that in control patients.

Heart rate and blood pressure. Heart rate did not change after acetylcholine injection in either group. Systolic blood pressure decreased significantly in the multivessel spasm group ($p < 0.01$) but not in the single-vessel spasm group during acetylcholine-induced spasm. The rate-pressure product tended to decrease after acetylcholine in the multivessel spasm group, but the change was not significant. It did not change after acetylcholine in the single-vessel spasm group. Myocardial lactate extraction ratio decreased significantly during acetylcholine-induced spasm in both groups ($p < 0.05$ and $p < 0.01$, respectively).

Discussion

Coronary effects of acetylcholine. Acetylcholine is an endothelium-dependent vasodilator (19) as well as a potent vasoconstrictor (20). Previous experimental studies (21–23) have shown that acetylcholine causes vasodilation in vessels with intact endothelium and that this vasodilative effect is abolished in vessels with impaired endothelium or with atherosclerotic changes. We have recently shown (24) that acetylcholine dilates the angiographically normal coronary artery in young humans, whereas it constricts both the angiographically normal human coronary artery in middle-aged humans and the atherosclerotic coronary artery in patients of all ages; these findings suggest that the effect of acetylcholine on the large epicardial coronary artery varies with the condition of the vascular endothelium (25,26).

It is also true that both experimental (27–29) and clinical studies (12–15) have shown that acetylcholine causes vasodilation in the coronary resistance vessels and increases the coronary blood flow, presumably through the release of endothelium-derived relaxing factor. The present study again demonstrated a transient and significant increase in coronary sinus blood flow after acetylcholine injection in control subjects without any significant changes in systemic

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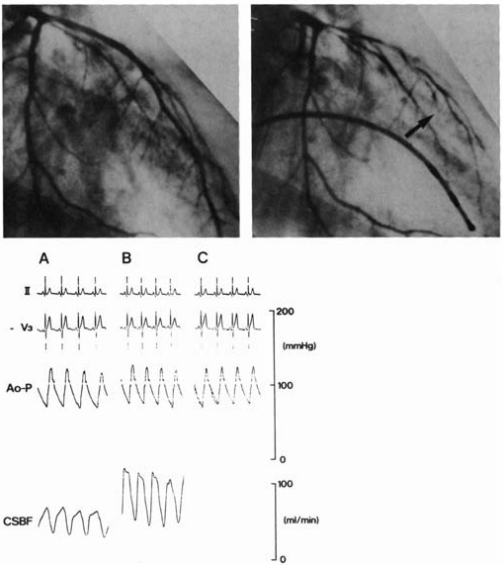


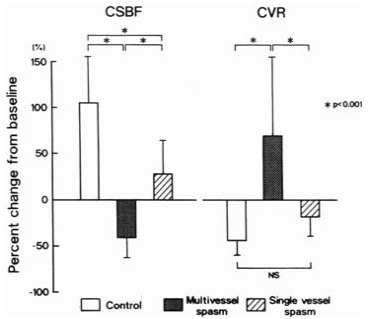
Figure 4. **Top,** Left coronary arteriograms taken before (**left**) and after (**right**) injection of 20 μg of acetylcholine in Patient 33 with single-vessel spasm. Total occlusion was induced in the midportion of the left anterior descending artery (**arrow**). **Bottom,** Electrocardiographic leads II and V_5, aortic pressure (Ao-P) and coronary sinus blood flow (CSBF) recorded at baseline (**A**) and 1 min after injection of acetylcholine (**B**) in the same patient. After injection of acetylcholine, mean coronary sinus blood flow increased from 55 to 88 ml/min. ST segment elevation in lead V_5 was noted just before arteriography (**C**).

hemodynamics, indicating acetylcholine-induced vasodilation at the level of the coronary resistance vessels.

Effects of acetylcholine on the conductance and resistance vessels in patients with variant angina. This study showed that intracoronary injection of acetylcholine induced spasm in the left coronary artery that was associated with signs of myocardial ischemia, including chest pain, ischemic ECG changes and myocardial lactate production in all of the patients with variant angina. Acetylcholine also induced a moderate degree of vasoconstriction in the "nonspasm" arteries of these patients. The large epicardial coronary artery of the patients with variant angina thus seems to have impaired vascular endothelium or a hyperreactivity to the vasoconstrictive effect of acetylcholine (10,11). The response of the "nonspasm" artery to constrictive stimuli is a matter of controversy (30): its constrictor response to ergonovine was shown to be similar to (31) or greater than (32) that in control subjects.

The most important finding of the present study was the significant increase in coronary sinus blood flow after acetylcholine injection despite the induction of spasm in the left coronary artery. The coronary sinus mainly drains the blood flow from the left coronary artery (33), and thus the increase

Figure 5. Percent changes in coronary sinus blood flow (CSBF) and coronary vascular resistance (CVR) after injection of acetylcholine in 10 control subjects and in 57 patients with variant angina (multivessel spasm, n = 10; single-vessel spasm, n = 47). See text for discussion.



in coronary sinus blood flow indicates an increase in left coronary artery flow. Because the rate-pressure product did not increase after acetylcholine injection but instead decreased, a primary vasodilation at the level of the coronary resistance vessels was induced by acetylcholine in the left coronary artery, presumably through the release of endothelium-derived relaxing factor. Thus, muscarinic receptor-mediated dilator response seems to be preserved in the vascular endothelium of the coronary resistance vessels, even in patients with variant angina.

How did acetylcholine increase coronary sinus blood flow while causing spasm in the epicardial coronary artery? Coronary sinus blood flow was not measured simultaneously with angiographic documentation of spasm but preceded it. Thus, it is possible that the vasodilative effect of acetylcholine on the coronary resistance vessels appeared before its constrictive effect on the epicardial conductance vessel; however, a significant reduction of coronary sinus blood flow was noted in the patients with multivessel spasm. Therefore, it is more likely that the constrictive effect of acetylcholine on the epicardial coronary artery was manifested almost simultaneously with its dilative effect on the resistance vessels. This does not imply that the coronary blood flow through the spastic artery increased after acetylcholine injection; rather, that the coronary blood flow through the "nonspasm" arteries increased, although vasoconstriction was also induced in these vessels. It has been reported that the maximal coronary flow response is maintained unless the lumen diameter of an epicardial coronary artery is reduced by >45% (34).

Single-vessel versus multivessel spasm. As clearly shown by the difference in the response to acetylcholine between the multivessel and single-vessel spasm groups, the outcome of the effect of acetylcholine on coronary sinus blood flow was largely related to the extent of myocardial ischemia induced. Thus, in the patients with a large ischemic region due to multivessel coronary spasm, the reduction in the coronary blood flow through the arteries with spasm was greater than the increase in the flow through the arteries without spasm, so that coronary sinus blood flow decreased. In contrast, in the patients with a relatively small ischemic region induced, the increase in blood flow through the nonspasm artery surpassed the reduction in the flow through the spastic artery, so that coronary sinus blood flow increased after acetylcholine injection. This was recognized in the majority of patients in the single-vessel spasm group; however, the increase in coronary sinus blood flow in this group was significantly less than that in control patients. It was unclear from the present study whether the vasodilator response to acetylcholine of the resistance vessels also occurred in the artery with spasm.

Ergonovine-induced spasm of the left coronary artery causes a decrease in both coronary sinus blood flow and great cardiac vein flow irrespective of the site and severity of spasm (9), findings that suggest an absence of vasodilative effect of the drug on the coronary resistance vessels. A recent clinical study (15) showed that the response of coronary vascular resistance to acetylcholine infusion was similar in normal control patients and patients with coronary artery disease but was significantly reduced in patients with hypercholesterolemia. In the present study most of the patients with variant angina had a normal serum cholesterol level.

Clinical implications. Variant angina is caused by the absolute reduction in the regional myocardial blood flow due to spasm of an epicardial coronary artery (1,2). Coronary sinus blood flow and oxygen saturation in the coronary sinus have been demonstrated to decrease before a spontaneous ischemic attack in patients with variant angina (3,4). As clearly shown in this study, the coronary blood flow in the total heart is not necessarily reduced when acetylcholine is used as a provocative agent for coronary spasm. Because the coronary vascular resistance decreases in the branches other than the artery with spasm during acetylcholine-induced spasm, a coronary steal to nonischemic regions is likely to develop (35), resulting in more severe myocardial ischemia when spasm is induced by acetylcholine.

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